

TITLE: Standard operating procedure (SOP) for the start-up, maintenance, sorting, and shutdown of the BD FACS Discover™ S8 Spectral Cell Sorter.

EFFECTIVE DATE: 4th March, 2026

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REVIEWED BY: Jodi Kroeger **(Signature)** **(Date)**

I. PURPOSE

This SOP describes the complete workflow required to operate the BD FACS Discover™ S8 Spectral Cell Sorter, including instrument startup, quality control, Accudrop, performing a sort, exporting data and images, and conducting a long-term shutdown. This procedure applies to all operators of BD FACS Discover™ S8 cell sorter.

II. REFERENCES

- **BD Biosciences.** (2025). *BD FACSDiscover™ S8 cell sorter with BD CellView™ Image Technology and BD SpectralFX™ Technology: User's guide* (Version 6.1.0).
- **Michael Kissner, CSCI Flow.** (2025). *The BDFACS Discover S8 Cell Sorter Image-Enabled Spectral Sorting.*
- <https://www.bdbiosciences.com/en-us/products/instruments/flow-cytometers/research-cell-sorters/bd-facsdiscover-s8?tab=overview>

III. MATERIALS AND REAGENTS

- BD FACS Discover™ S8 Spectral Cell Sorter
- FACSFlow Sheath (or similar)
- Clorox bleach
- Deionized water
- 70% ethanol
- 5% BD Detergent solution (660585)
- FACSDiscover™ Setup Beads (665056)
- CellView™ Calibration Beads (665055)
- BD FACS™ Accudrop RUO beads (661612)
- Koolance® High-Performance Liquid Coolant (LIQ-702CL-B)
- 5mL test tubes

IV. PROCEDURE

4.1. Safety Considerations

- Always wear appropriate PPE.
- All sample preparation and handling must occur under a biosafety cabinet.
- Handle bleach, ethanol, and waste following institutional chemical-safety procedures.

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- Disconnect quick-connects carefully to prevent pressure hazards or spills.

4.2. Instrument Start up, Daily QC, and Sort-Setup

1. Power on the computer located beneath the biosafety cabinet.
2. After 60 seconds, press power button on the right side of the S8 instrument.
3. Log into Windows using posted username and password.
4. Launch **FACSCorus** software and log in.
5. Roll the fluidics cart out and ensure no lines are caught under the wheels.
6. Check fluidics containers:
 - Empty waste if full and fill bottom with ~1-inch undiluted bleach.
 - Refill sheath to weld line. (Typically filled after previous sort.)
 - Fill DI water tank to green tape line.



7. Roll cart back under the biosafety cabinet and engage parking brake.
8. In **FACSCorus** software, click on **Cytometer** and select **System Start Up**.
9. Select **Extended Fluidics Startup** and follow prompts carefully.

1

Initiate extended fluidics startup

Start

2

Insert the closed loop nozzle

3

Prepare the tanks and tubing connections for the extended cleaning cycle

- Fill the DI water bottle with 1.2L of sterile DI water
- Bypass the sheath filter by disconnecting the sheath line connectors from the filter and connecting them together
- Disconnect the sheath line from the sheath tank and connect it to the Cleaning port on the fluid storage cart
- Empty the waste tank

4

Prepare the tanks and tubing connections for operation

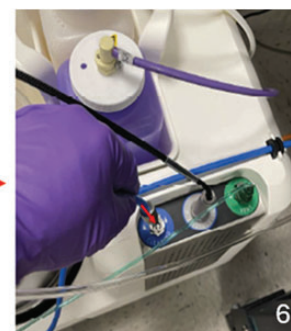
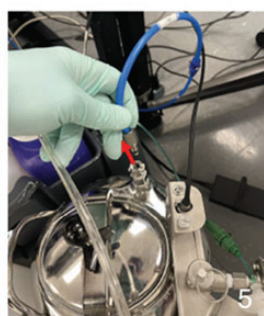
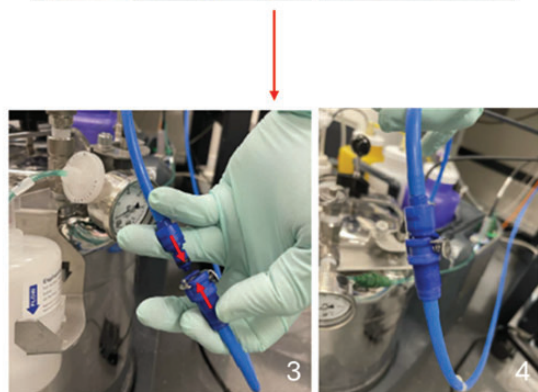
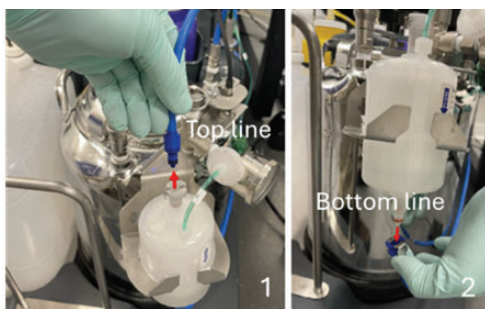
- Fill the sheath tank with sterile, 1X PBS
- Disconnect the sheath line from the Cleaning port and reconnect to the sheath tank
- Re-install the sheath filter into the sheath line
- Empty the waste tank

5

Start sheath filter purge and prime the fluidics

10. If the blue sheath line is connected to the cleaning port, proceed with startup.

11. If blue line is connected to the white sheath filter, disconnect both quick-connects from filter and temporarily connect the two lines together per startup instructions (sequence pictured below). Click ‘Continue.’



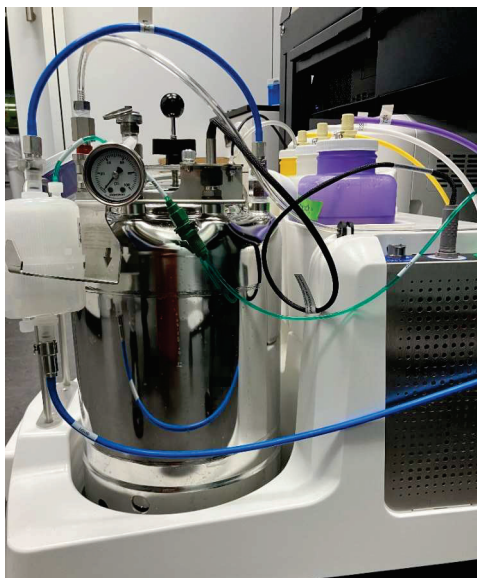
Images derived from BD
FACS Discover™ S8 User's
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12. Restore connections to white sheath filter and sheath tank when prompted by the startup wizard (see image below).



13. Click '**Close**' once the startup wizard completes.
14. Open the sliding door on sorter, remove closed-loop nozzle from bottom of the flow cell cuvette and place it in the nozzle holder.
15. Insert appropriate sorting nozzle and secure in place with locking lever.
16. Turn on the stream and wait for stabilization.
17. Run 1–2 minutes each of BD detergent followed by DI at max flow rate of 50.
18. Prepare Discover S8 QC beads (665056) and CellView Imaging QC beads (665055):
 - 2 drops into 500 μ L filtered 1 $\times$ PBS.
19. Go to **System Start Up** and skip previously completed tasks to access **QC**.
20. Load Discover S8 QC beads and close the stage door. Run QC.
21. When prompted, load CellView beads and run imaging QC.
22. Load S8-specific Accudrop beads (661612) and follow workflow prompts to complete Accudrop sort setup.
23. Review QC and Accudrop reports to verify instrument performance. Respond to warning and/or failures accordingly. Reference: **BD FACSDiscover™ S8 cell sorter User's Guide (ver. 6.1.0), Section 12: Troubleshooting.**
24. Run 2 minutes BD detergent followed by 2 minutes DI water.
25. Sterile sort prep: Run 15 minutes of 70% ethanol, followed by 10 minutes of sterile 1X PBS on max flow rate of 50.

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4.2. Performing a Bulk Tube Sort

1. New experiment setup: Navigate to the **Experiments** tab and select **New Experiment**.
2. To import an existing experiment template, select '**Import**'.
3. Name the experiment and add panel information accordingly. Assign imaging markers if applicable.
4. Use a fully stained sample to optimize gain settings and set the **Region of Analysis (ROA)**.
5. If using a combination of beads and cells for unmixing, reset ROA for beads before recording.
6. **Record** single-color controls.
7. Create an unmixing matrix in **Matrix Previewer**.
8. Apply the matrix to all samples via **Set Up Matrix** in the **Sample Manager**.
9. In **Sort Set Up** tab, select tube type, number of tubes, targets of interest, and purity mode.
10. Install the appropriate tube adapter and connect recirculator lines.
11. Place empty stream alignment tubes of the same size as the intended collection tubes and install the splash guard assembly.
12. Perform **Side Stream Deflection**: Adjust with arrow keys as needed; confirm drops fall into tubes at the correct angle.

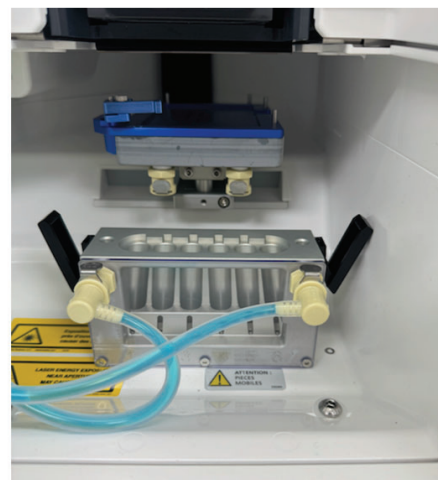


Image courtesy of Columbia

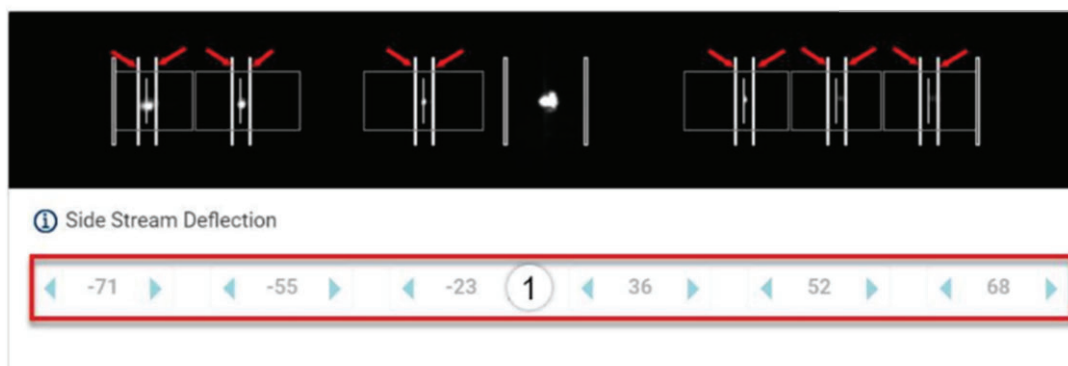


Image derived from BD FACS
Discover™ S8 User's Guide

13. Remove stream alignment tubes and place labeled collection tube(s) into the target-assigned position(s).
14. **Load** sample, adjust flow rate as necessary, and click '**Sort**'.

4.3 Performing a 96-well Plate Sort

15. In the **Sort Set Up** tab, select appropriate plate from drop down menu.

16. Select **Enable Index Sort**, if necessary.

COLLECTION SETUP

Device Type: Plate ☐ Enable Index Sort

BD-Defined Plate: 96 well

Plate Name: Default [Optimize Plate](#)

Sort Mode: Single Cell

17. Assign sort populations to wells.

SORT SETUP

Assign a sort population by clicking any combination of wells and selecting the population and number of events that you want.

Unassign Selected

Select All

Initial Buffer Volume: 0.00 mL

Number of Events: 1

Max: 44354 events

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	1	1	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1	1	1	1	1	1
G	1	1	1	1	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1	1	1	1	1

Population Hierarchy

All Events

Saturated

Unsaturated

Scatter

SSC Singlets

FSC Singlets

Live

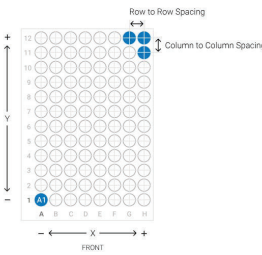
mCherry+

18. Select **Optimize Plate** to aim the stream and follow the wizard prompts.

Optimize Plate

Name
Plate 1

BD-Defined Plate
96 well



✔ Splash shield installed
✔ Sort collection chamber door closed

- 1

Install the splash shield

Replace any collection device in the sort collection chamber with the splash shield.
- 2

Close the sort collection chamber door
- 3

Load the plate onto the stage

1. Click Eject. Load the plate on the stage so the A1 well sits at the front-left of the stage.

2. Close the sort collection chamber door.

Eject
- 4

Test sort into the four corner wells using sheath fluid

1. Click Test Sort.

2. Wait until the plate automatically ejects after the last deposition is complete.

3. Check the position of the drops on the plate. If the drops are accurate, close this window. Otherwise, optimize the plate (step 5).

Test Sort 4 Corners

Test Sort Entire Plate
- 5

Optimize the plate

1. Looking at the drops on the plate, adjust the A1 Offset Position using the X and Y input fields to move the stage.

2. Wipe off the drops from the plate then close the sort collection chamber door.

3. If necessary, adjust the Row to Row and Column to Column Spacing using the input fields.

4. Click Test Sort (step 4) to check the alignment.

5. If needed, click Restore to start from default.

A1 Offset Position: X mm

Y mm

Row to Row Spacing: mm

Column to Column Spacing: mm

Restore to Default

Click Retract if you need to move the stage back.

Retract

Close

Save Plate

19. Click **Save Plate**.

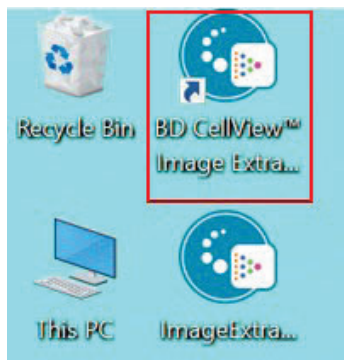
20. Proceed with sort.

4.4 Exporting Data and Image Files

21. Go to **View Reports** and export each sort report.

22. To export .fcs files and raw image files (.cvw) to the computer desktop, navigate to **View Data** tab, click '**Export**'. Note: Uncheck unnecessary statistics to reduce the file size.

23. Use CellView Image Extractor to convert .cvw to .tiff; do not modify folder structure.

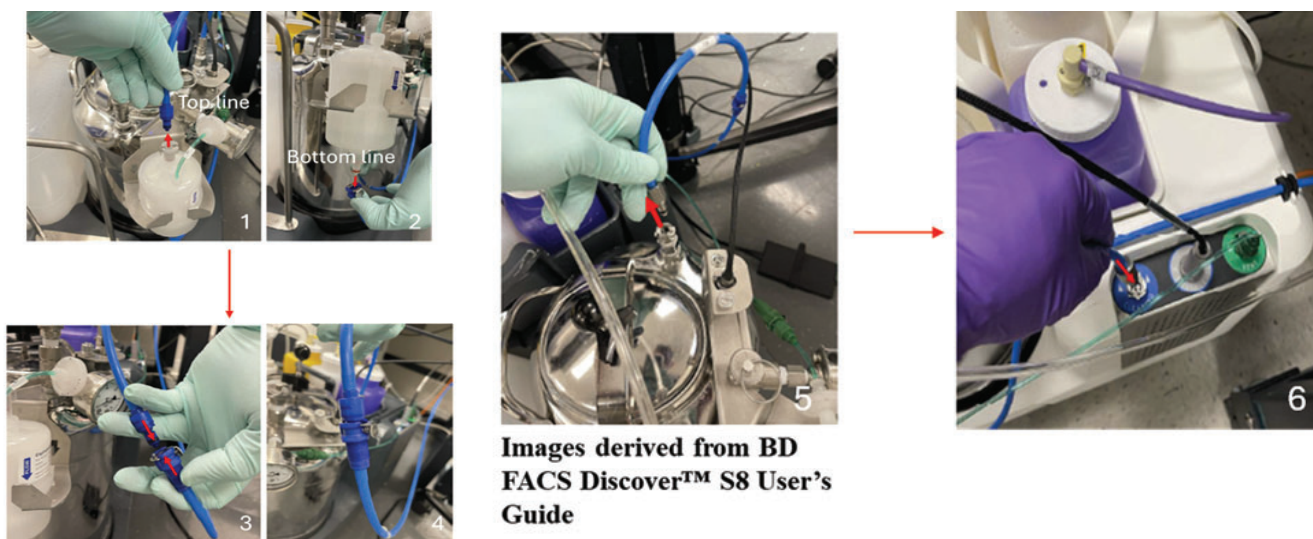


24. Transfer the experiment folder to the Flowlab directory.

Note: Instrument shutdown can be done during large data transfers.

4.5 Instrument Shutdown (Long Term)

25. Using an open experiment, run in order for one (1) minute each at a flow rate of 50:
 - BD detergent
 - 10% bleach
 - DI water
26. Turn off stream.
27. Remove and store sort nozzle in a 15mL conical tube with DI water.
28. Install closed-loop nozzle.
29. Perform three (3) **Clean Flow Cell** cycles with BD detergent.
30. Refill ethanol bottle to the green tape line using 70% ethanol.
31. Refill sheath tank:
 - Disconnect airline quick-connect.
 - Depressurize tank by pulling up on the pin.
 - Refill with FACSFlow to the weld line.
 - Reconnect airline quick-connect securely.
32. Select **Long Term Shutdown** in the wizard.
33. When prompted, disconnect blue sheath lines from filter and plug into cleaning port as follows:



34. Select '**Continue**' on **Extended Shutdown** wizard.
35. When prompted, install a tube of 70% ethanol and click **Continue**.
36. Exit software.
37. Shut down computer.
38. Roll cart under cabinet.

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Standard Operating Procedure

39. Power off recirculator.
40. Press silver button to power off lasers.

V. REVISION HISTORY

4 th March, 2026
